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Inhibition by FK506 of established lesions of collagen-induced arthritis in rats

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SUMMARY

We investigated the superior potency of the immunosuppressive agent FK506 on collagen-induced arthritis in rats. In our initial studies, we demonstrated that only one shot administration of FK506 at a dose of 10 mg/kg on the same day as type II collagen immunization suppressed the incidence of arthritis completely as well as humoral and delayed-type hypersensitivity (DTH) skin test responses to type II collagen. Yet no major side effects were observed in the rats treated with such a high dose of FK506. Additional studies demonstrated that pretreatment with FK506 on day −7 or day −3 was effective in suppressing the severity of arthritis and immune responses to type II collagen. The immunosuppressive effect of a single high-dose administration of FK506 continued for at least 1 week in this animal model of arthritis. A single administration of FK506 at a dose of 10 mg/kg on day 12 or 15, after the clinical onset of arthritis, was also effective in suppressing the severity of arthritis and immune response to type II collagen. We conclude that FK506, in this model, possesses an important, curative action when applied therapeutically. The outlook of FK506 treatment in clinical autoimmunity is promising at present.

Keywords FK506 collagen arthritis immunosuppression established lesion therapeutic effect

INTRODUCTION

A structurally novel macrolide FK506, isolated from the fermentation broth of a strain of soil fungus, *Streptomyces tsukubaensis* (Kino *et al.*, 1987a), has recently been demonstrated to have potent immunosuppressive activity at concentrations several hundredfold lower than cyclosporin A (CyA) (Kino *et al.*, 1987c; Sawada *et al.*, 1987; Kay *et al.*, 1989). FK506 represents a great advance in immunosuppression with few undesirable side effects (Starzl *et al.*, 1989; Todo *et al.*, 1989) such as nephrotoxicity, bone marrow suppression and infections. The immunosuppressive action of this drug appears to depend on its ability to modify the activation of helper T cells, although its precise mode of action is not understood. In *in vitro* experiments, FK506 has been shown to inhibit interleukin-2 (IL-2) receptor expression, mixed lymphocyte culture responses (MLR), cytotoxic T cell generation and production of T cell-derived soluble factors such as IL-2, interleukin-3 (IL-3), and interferon-gamma (IFN-γ) (Kino *et al.*, 1987b). Furthermore, it has permitted successful allograft transplantation of heterotopic hearts (Lim, Thiru & White, 1987; Murase *et al.*, 1987), limbs (Arai *et al.*, 1989) or skin (Inamura *et al.*, 1988c) in rats; kidneys (Todo *et al.*, 1987a) or livers (Todo *et al.*, 1987b) in dogs; and kidneys in subhuman primates (Todo *et al.*, 1989). However, there have been very few studies concerning the influence of FK506 on experimental autoimmune diseases (Inamura *et al.*, 1988a, 1988b; Takabayashi *et al.*, 1989). Inamura *et al.* (1988a) showed that FK506 treatment at a dose of 3·2 mg/kg per day was very effective in suppressing collagen-induced arthritis in rats when administered from days 0 to 4, but ineffective when administered from days 7 to 11. FK506, however, is now used in clinical transplantation to prevent rejection (Starzl *et al.*, 1989). As a result of this interest, and the paucity of studies concerning the effect of FK506 on immune response in experimental autoimmune disease models, we decided to investigate the influence of FK506 administration on collagen-induced arthritis in rats.

We first determined that under the *in vivo* conditions employed, a single high-dose treatment with FK506 on the day of type II collagen immunization would suppress collagen arthritis. Then the drug was administered at all stages of the disease process of collagen arthritis and its effects on incidence and severity of arthritis as well as on immune responses to type II collagen were examined.

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MATERIALS AND METHODS

Animals

Outbred female Sprague-Dawley rats were purchased from Charles River Japan (Kanagawa, Japan). They were allowed 1

week to adapt to their environment and were used aged 5–6 weeks, weighing 130–150 g at the start of the experiment. All the animals received standard laboratory chow and water *ad libitum*.

Preparation of type II collagen and production of collagen arthritis

Type II collagen was isolated and purified from bovine articular cartilage, as previously described (Trentham, Townes & Kang, 1977). The purity was assessed as described elsewhere (Kaibara *et al.*, 1983). Lyophilized type II collagen was dissolved in 0·1 M acetic acid at a concentration of 3 mg/ml. Equal volumes of collagen solution and Freund's incomplete adjuvant (Difco Laboratories, Detroit, MI) were emulsified, using a homogenizer (Polytron Pt 10-35; Kinematica, Lucerne, Switzerland), and kept cold on an ice bath. Collagen arthritis was produced by intradermal injection of 1 ml of cold emulsion at several sites on the back and at one or two sites at the base of the tail on day 0. In our hands, 80–90% of the rats consistently developed arthritis with this regimen of immunization (Kaibara *et al.*, 1983; Takagishi *et al.*, 1986; Arita *et al.*, 1987).

Treatment with FK506

FK506 (FR900506; Fujisawa Pharmaceutical Co., Osaka, Japan) was provided in crystalline powder form (molecular weight 822) and dissolved in physiological saline at a concentration of 5 mg/ml. FK506 was administered subcutaneously under light ether anaesthesia on the day and at the doses indicated. The amount of the drug was adjusted according to the body weight of the recipient on the day of drug administration. Control rats were immunized with type II collagen and received no FK506 treatment. FK506-treated rats and control rats were handled identically.

Assessment of arthritis

Rats were examined daily for 28 days after immunization with type II collagen to record the day of onset and the severity of arthritis. The lesions of the four paws were each graded from 0 to 4 according to the increasing extent of periarticular erythema and swelling as well as joint deformity, as described previously (Kaibara *et al.*, 1983). The maximum possible score was 16.

Immune response to type II collagen

DTH skin testing was performed as described by Griffiths *et al.* (1981), using 50 μg of type II collagen in 0·05 M Tris-HCl buffer, pH 7·4, containing 0·2 M NaCl. The response was read at 48 h.

Serum antibodies to type II collagen were measured by ELISA as described elsewhere (Kaibara *et al.*, 1983), adapted from the method of Voller, Bidwell & Bartlett (1976). The quantity of IgG antitype II collagen antibody was expressed as milligrams per 100 ml serum by comparison with standard curves obtained from an affinity-purified rat antitype II collagen antibody control (Kaibara *et al.*, 1984).

Pathological studies

Rats were killed after the 4-week experimental period for histological examination of the side-effects of the drug. Heart, lung, thymus, liver, spleen, kidney and adrenal gland were removed and complete post mortem examination was performed. Blocks of tissue were fixed in 15% neutral buffered formalin and processed to paraffin wax. Sections were stained with haematoxylin and eosin. All tissues were examined blindly without knowledge of the immunosuppressive therapy.

Statistical analysis

Continuous variables were analysed by group means (Student's *t*-test), and dichotomous variables by their proportionate group frequencies (χ^2 test). $P < 0.05$ was considered statistically significant.

RESULTS

Body weight

There was a mean increase in body weight of 29·6±6·1% in untreated control animals over the 14-day course in the experiment. In comparison with controls, there were not significant reductions in weight gain in FK506-treated groups (30·2±5·1%).

Dose–response studies of FK506 on the development of collagen arthritis in rats

Dose–response studies of a single-dose treatment with FK506 were carried out, in which the rats received FK506 on the day of

Table 1. Treatment of collagen-induced arthritis with FK506 on the day of immunization

Dose (mg/kg)	Incidence of arthritis		Day of onset*	Arthritic index†	Antibody level‡		DTH§
	n	%			Day 14	Day 21	
2·5	6/11	55	12·7±0·3¶	2·5±0·7**	12·7±1·7¶	20·4±4·5¶	6·3±0·3
5·0	4/10††	40	11·8±0·2¶	1·6±0·7¶	4·9±1·8¶	4·7±1·8¶	4·7±0·6††
10·0	0/10¶	0	—	0¶	0·3±0·1¶	0·2±0·1¶	0·7±0·4¶
Control	14/16	88	9·4±0·2	6·4±0·7	48·6±2·7	113·8±8·2	5·9±0·2

Groups of rats were immunized with 1·5 mg of type II collagen and treated with s.c. FK506 on day 0.

*Based on arthritic rats only (mean±s.e.m.).

†Expressed as the mean of maximum arthritic indices±s.e.m.

‡Antibody levels to type II collagen were measured by ELISA on days 14 and 21 and expressed as mg of IgG anti-collagen antibody/100 ml of serum (mean±s.e.m.).

§Performed on day 25 and expressed as the mean±s.e.m. diameter of induration (mm).

¶ $P < 0.001$; ** $P < 0.01$; †† $P < 0.05$ *versus* control group.

Table 2. Pretreatment of collagen-induced arthritis with FK506 (10 mg/kg)

Injection	Incidence of arthritis		Day of onset	Arthritic index	Antibody level		DTH
	n	%			Day 14	Day 21	
Day −15	10/14	71	12·9±0·7*	4·1±0·9	49·2±9·0	63·9±9·5*	4·1±0·7†
Day −7	3/15*	20	12·3±0·5*	1·1±0·6*	21·1±4·5*	33·8±6·4*	3·9±0·4*
Day −3	0/10*	0	- -	0*	5·5±2·4*	31·6±12·3*	3·4±0·4*
Control	14/16	88	9·4±0·2	6·4±0·7	48·6±2·7	113·8±8·2	5·9±0·2

Groups of rats were immunized with 1·5 mg of type II collagen on day 0 and treated with FK506 (10 mg/kg, subcutaneously). Parameters and units are as in Table 1.
* $P<0.001$; † $P<0.05$ *versus* control group.

Table 3. Prophylactic treatment of collagen-induced arthritis with FK506 (10 mg/kg)

Injection	Incidence of arthritis		Day of onset	Arthritic index	Antibody level		DTH
	n	%			Day 14	Day 21	
Day 0	0/10*	0	—	0*	0·3±0·1*	0·2±0·1*	0·7±0·4*
Day 3	4/10†	40	11·0±0·5‡	1·8±0·4*	4·6±1·2*	6·1±3·9*	5·0±0·4†
Day 6	3/10†	30	9·3±0·3	2·1±1·0‡	38·2±5·2	29·8±4·1*	3·3±0·7*
Control	14/16	88	9·4±0·2	6·4±0·7	48·6±2·7	113·8±8·2	5·9±0·2

Groups of rats were immunized with 1·5 mg of type II collagen on day 0 and treated with FK506 (10 mg/kg, subcutaneously). Parameters and units are as in Table 1.
* $P<0.001$; † $P<0.05$; ‡ $P<0.01$ *versus* control group

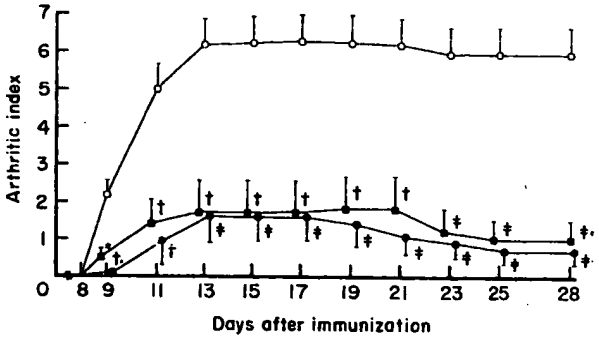

Fig. 1. Time course of the severity of arthritis, expressed as the mean arthritic indices ± s.e.m., for the rats treated with FK506 (10 mg/kg) prophylactically. (●) FK506-treated (day 3) rats; (■) FK506-treated (day 6) rats; and (○) control rats. * $P<0.05$; † $P<0.01$; and ‡ $P<0.001$ *versus* control group.

type II collagen immunization (Table 1). An inflammatory polyarthritis was induced in 14 out of 16 control rats immunized with type II collagen with no FK506 treatment. FK506 treatment at a dose of 5 or 10 mg/kg on the day of immunization produced significant suppression of arthritis induction during an observation period of 28 days, whereas a dose of 2·5 mg/kg of FK506 was ineffective. Serum antibody levels to type II collagen were measured on days 14 and 21, and DTH skin testing was performed on day 25. The control rats showed high antibody levels and strong positive skin test responses to type II collagen, whereas very weak antibody responses and weak skin test responses to type II collagen could be detected in the rats treated with 5 or 10 mg/kg of FK506.

Effect of pretreatment with FK506 on collagen arthritis in rats

Having established that FK506 treatment at a dose of 10 mg/kg on the day of type II collagen immunization completely suppressed the development of arthritis as well as humoral and DTH skin test responses to type II collagen, we then studied the effect of FK506 at all stages of the disease process in collagen arthritis. In this section, we wished to see whether pretreatment with FK506 before the type II collagen immunization would have similar effects (Table 2). Groups of rats were treated with 10 mg/kg of FK506 on day −15, day −7, or day −3 and were immunized with type II collagen on day 0. As shown in Table 2, FK506 treatment on day −7 or −3 (given prior to immunization of rats with type II collagen) resulted in significant suppression of clinical symptoms of arthritis and immune responses to type II collagen. In the group of rats pretreated with FK506 on day −15, the incidence and the severity of arthritis were not suppressed significantly.

Effect of prophylactic treatment with FK506 on collagen arthritis in rats

In this section, groups of rats were administered FK506 at a dose of 10 mg/kg during the induction phase of arthritis (Table

Table 4. Treatment of established lesion of collagen-induced arthritis with FK506 (10 mg/kg)

Injection	Incidence of arthritis		Day of onset	Arthritic index	Antibody level		DTH
	n	%			Day 14	Day 21	
Day 9	12/20	60	9·3±0·1	3·8±0·8*	44·4±5·3	55·5±8·6†	4·7±0·5*
Day 12	17/21	81	10·2±0·2	6·3±0·7	39·3±4·1	26·2±6·9†	6·0±0·2
Day 15	9/10	90	9·1±0·1	6·2±0·7	43·6±7·1	46·2±8·7†	5·1±0·4
Day 18	9/10	90	9·3±0·2	7·0±0·8	50·7±6·7	79·5±14·7*	6·3±0·2
Control	14/16	88	9·4±0·2	6·4±0·7	48·6±2·7	113·8±8·2	5·9±0·2

Groups of rats were immunized with 1·5 mg of type II collagen on day 0 and treated with FK506 (10 mg/kg, subcutaneously). Parameters and units are as in Table 1.
* $P<0.05$; † $P<0.001$ *versus* control group.

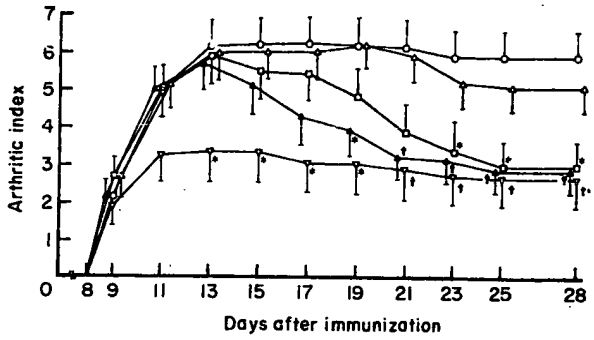

Fig. 2. Time course of the severity of arthritis, expressed as the mean arthritic indices ± s.e.m., for the rats treated with FK506 (10 mg/kg) therapeutically. (▽) FK506-treated (day 9) rats; (▲) FK506-treated (day 12) rats; (□) FK506-treated (day 15) rats; (△) FK506-treated (day 18) rats; and (○) control rats. * $P<0.05$; † $P<0.01$ *versus* control group.

3, Fig. 1). The development of arthritis was significantly suppressed in the animals treated with FK506 on day 3 or day 6. These regimens were very effective in inhibiting the swelling of the paws as well as the formation of anti-type II collagen antibodies and DTH response to type II collagen.

Effect of therapeutic treatment with FK506 on the established lesion of collagen arthritis in rats

Since more than a half of the animals in the control group developed arthritis on day 9 in our hands, the drug treatment on day 9 or later is considered to affect the established lesion of arthritis. The response of the established disease to therapeutic treatment with FK506 at a dose of 10 mg/kg is shown in Table 4 and Fig. 2. In the FK506-treated group on day 9, the incidence of arthritis and disease onset were not affected, compared with those of the control group. In this group the severity of arthritis was reduced gradually and suppressed significantly compared with the control group from days 13 to 28. The antibody response on day 21 and DTH skin test responses to type II collagen were significantly suppressed in the rats with FK506 administration n day 9. When FK506 was administered n day 12, the severity of arthritis was suppressed from days 19 to 28. In the FK506-treated group on day 12, the antibody response to type II collagen on day 21 was significantly suppressed but the DTH response on day 25 was not affected. High-dose treatment with FK506 even on day 15 altered the time course of severity of arthritis at the end of the observation period from days 23 to 28, but FK506 administration on day 18 did not alter the severity of arthritis significantly. In addition to the prophylactic effect, FK506 also showed a strong therapeutic effect in the groups of rats with FK506 administration after the onset of arthritis.

Pathological studies

Gross abnormalities were not found at autopsy. No abnormalities were observed in any of the tissues examined in all of the experimental groups.

DISCUSSION

While a variety of immunosuppressive agents or anti-inflammatory drugs has been used for the treatment of collagen-induced arthritis in rats or mice, we are aware of no reports concerning the successful therapeutic effect of these drugs on the established lesion of collagen arthritis (Sloboda *et al.*, 1981; Stuart *et al.*, 1981; Phadke, Carroll & Nanda, 1982; Probert, Schrier & Gilbertsen, 1984; Arita *et al.*, 1987). Particularly, CyA, a powerful immunosuppressive drug widely used in the treatment of autoimmune diseases or allograft rejection, has no suppressing but enhancing effect on collagen-induced arthritis in rats when administered soon after its clinical onset (Kaibara *et al.*, 1983). The present study demonstrates a remarkable immunosuppressive potency of the newly discovered macrolide antibiotic, FK506, on this animal model of arthritis. A single high-dose treatment with FK506 at a dose of 10 mg/kg resulted in significant suppression of ongoing and established lesion of collagen arthritis.

In earlier work, Inamura *et al.* (1988a) demonstrated that FK506 at a dose of 3·2 mg/kg per day was very effective in suppressing arthritis when given during the afferent limb of the immune response to type II collagen (from day 0 to day 4), whereas the drug was only marginally effective when given during the efferent limb of the immune response (from day 7 to day 11). The results of the present study showed that a single high-dose treatment with FK506 at a dose of 10 mg/kg from day 3 to day 15 was effective in suppressing the ongoing immune response as well as in decreasing the manifestations of this arthritis. This observation suggests that FK506 is capable of

suppressing not only the afferent limb of the immune response but also the efferent limb of the immune reaction. The exact explanation of the difference between the results of these two studies is not clear, but daily treatment with FK506 at a dose of 3·2 mg/kg from day 7 to day 10 may have been inadequate to suppress the ongoing immune response in this animal model of arthritis.

Our findings are consistent with the recent reports that, under certain conditions, FK506 can inhibit the cardiac allograft rejection in rats (Ochiai *et al.*, 1987) and the delayed rejection of renal transplantation in baboons (Todo *et al.*, 1989). We have also reported that successful treatment to prevent or to reverse rejection of limb allografts was achieved with a single treatment with FK506 (10 mg/kg) on day 7 or 10 while the untreated limb allografts were anticipated to have clinical signs of severe rejection within 6 days after operation (Arai *et al.*, 1989).

The exact mechanism of action of FK506 is not totally understood, but its immunosuppressive properties have been reported to include suppression of MLR, IL-2 receptor expression and production of T cell-derived soluble factors such as IL-2, IL-3, and IFN-γ, which are released by activated T cells with the antigenic stimulation (Kino *et al.*, 1987b, 1987c). Kino *et al.* (1987b) suggested that FK506 may promote tolerance induction by sparing IL-3-dependent suppressor cells in a similar way to CyA. FK506, however, does not reduce the paw oedema in acute inflammatory models (Inamura *et al.*, 1988a).

In the present experiment the antibody response was allowed to establish before FK506 administration in the groups of rats with FK506 treatment on day 12 or 15, but there was a pronounced reduction in the levels of circulating anti-type II collagen antibodies on day 21. It is conceivable that in this model FK506 could inhibit not only early activation process in T cells and/or macrophages providing the stimulus for B cell activation or proliferation of B cells, but also activated T cells, the *in vivo* activity of which cannot be directly affected by CyA. Furthermore, the suppressive effect of FK506 treatment at a dose of 10 mg/kg on day 15 on the clinical symptoms of collagen-induced arthritis suggests that FK506 is capable of suppressing not only the ongoing antibody production, but also the inflammatory cell mediators triggered by anti-type II collagen antibodies, the complement cascade or the cells that are responsible for the maintenance of the arthritis.

Drug toxicity may prove to be a limiting factor in the use of immunosuppressive agents. Here, although we used a relatively high dose (10 mg/kg) of FK506, which was administered only once, animals treated with this dose gained weight normally and did not die at the end of the experiment. There is little detailed published information concerning the toxicology of FK506 (Nalesnik *et al.*, 1987; Thiru, Collier & Calne, 1987; Stephen *et al.*, 1989) and the toxicity of FK506 has been suggested to be species-specific (Todo *et al.*, 1988a). In the present study, the histological examination did not show arteritis in all organs examined, thymic medullary atrophy, nor proximal tubular cell vacuolation in the kidney. These observations are in agreement with the recent findings by Nalesnik *et al.* (1987) and Todo *et al.* (1988b) that it is comparatively safe in rats, monkeys and baboons. Starzl *et al.* (1989), in their first clinical trials of FK506 for liver, kidney and pancreas transplantation concluded that it was very potent and free of serious side-effects in humans. Taken together, FK506 will prove useful as a clinical immunosuppressive agent, perhaps as an adjunct to established forms of therapy.

We have demonstrated that a single high-dose treatment with FK506 provided a successful treatment of the established lesion of collagen arthritis with no severe toxic effect.

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CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY 46, 82–90 (1988)

Immunosuppressive Effect of FK506 on Collagen-Induced Arthritis in Rats

NORIAKI INAMURA,[1] MICHIZANE HASHIMOTO, KUNIO NAKAHARA, HATSUO AOKI, ISAMU YAMAGUCHI, AND MASANOBU KOHSAKA

Exploratory Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Ibaraki, Japan

FK506, a new immunosuppressive agent, was given intramuscularly to rats for 12 days, starting on the day of type II collagen immunization. FK506 in doses of 0.32 mg/kg or more suppressed arthritis and also suppressed humoral and skin test response to type II collagen. FK506 suppressed arthritis only when given during the afferent limbs of immune response (0–4 days), whereas the drug was only marginally effective when treatment was started during the efferent limbs of immune response (7–11 days). FK506-induced immunosuppression continued and/or was maintained throughout the experiments (50 days). These rats immunized with type II collagen and treated with FK506 failed to develop arthritis even following a secondary immunization 50 days later but were fully capable of developing experimental allergic encephalomyelitis. This result suggest that FK506-treated rats develop specific unresponsiveness toward the type II collagen. It is concluded that FK506 is a strong immunosuppressive drug on collagen-induced arthritis.

INTRODUCTION

Disorders of the immune system are undoubtedly responsible for human rheumatoid arthritis (RA).[2] The demonstration of antibodies to collagen in the sera and synovial fluids of patients with RA (1–8) has led to the view that immunologic hypersensitivity to collagen may, at least in part, contribute to the inflammation and joint destruction that is observed in the disease. Supporting this view, several studies (9–13) have shown that immunization with heterologous or homologous native type II collagen could induce an inflammatory polyarthritis. Since collagen-related autoimmunity has been described in patients with RA (1–8), the same pathophysiologic mechanisms responsible for collagen arthritis might cause some of the lesions of RA (14). Additional evidence supporting an immune-mediated pathogenesis of collagen arthritis includes suppression of arthritis treated with immunosuppressive agents (15–17).

FK506 is a new immunosuppressive agent, extracted from the fermentation broth of *Streptomyces tsukubaensis* (29). The chemical structure of FK506 belongs to Macrolides as shown in Fig. 1 and is entirely different from that of cyclo-

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[2] Abbreviations used: MLR, mixed-lymphocyte reaction; RA, rheumatoid arthritis; ICFA, incomplete Freund's adjuvant; id, intradermal(ly); im, intramuscular(ly); ELISA, enzyle-linked immunosorbent assay; PBS, phosphate-buffered saline; OPD, orthophenylenediamine; DTH, delayed-type hypersensitivity; MBP, myelin basic protein; EAE, experimental allergic encephalomyelitis; CFA, complete Freund's adjuvant; CsA, cyclosporine.

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FK506 on the development of c
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FIG.

sporine (18). Recent studies have shown that FK506 *in vitro* inhibited mixed-lymphocyte reaction (MLR) responses and the induction of cytolytic lymphocytes in these cultures by interfering with the action and/or production of lymphokines during the process of T-cell activation and proliferation without any significant myelotoxicity. These *in vitro* effects were highly potent than cyclosporine (30). The profound immunosuppressive properties of FK506 were subsequently explored in experimental transplantation, and could prolong skin graft survival in rat [19], indefinitely prolong survival of cardiac allografts in rats (20, 21), and prolong the life of canine kidney transplants to 200 days (22).

In the present study, we evaluated the effect of short-term treatment with FK506 on the development of collagen arthritis and on induction of unresponsiveness to collagen arthritis.

MATERIALS AND METHODS

Animals. Inbred female Lewis rats were purchased from Charles River Japan, Atsugi, Kanagawa. They were handled under specific pathogen-free conditions and acclimated to the new environment for a week before use.

Immunization procedures. Type II collagen was purchased from Maruzen Oil Biochemical, Tokyo, Japan, and dissolved overnight at 4°C in 0.01 *M* acetic acid at a concentration of 2 mg ml. The solution was emulsified in an equal volume of incomplete Freund's adjuvant (ICFA, Difco Laboratories, Detroit, MI). Each rat was immunized with 0.5 ml of the cold emulsion by several intradermal (id) injections on the back and one or two injections into the base of the tail.

Treatment with FK506. FK506 containing HCO-60 and D-mannitol was suspended in saline. The placebo preparation of the same formulation without FK506 was used as the control. These suspensions were injected intramuscularly (im) at various doses in 0.4 ml/kg on the days indicated in the text.

Assessment of arthritis. Rats were examined daily. The onset of arthritis was defined as the time when the first signs of erythema and swelling of one or more limbs appeared. The volume of both hind legs below the knee joint was measured with a plethysmometer (Ugo Basile, Comero-Varese, Italy).

Immunoassay of antibody to type II collagen. Sera from rats were obtained by

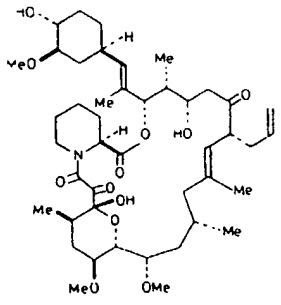

FIG. 1. Structure of FK506.

retroorbital bleeding using a capillary tube. Anti-collage antibody was measured by an enzyme-linked immunosorbent assay (ELISA) system described by Kaibara *et al.* (15), Stuart *et al.* (23), and Kerwar *et al.* (24) with minor modifications. Briefly, each well of the flat bottomed micro-ELISA plates (Dynatech Laboratories, Alexandria, VA) was incubated overnight at room temperature with 200 μl of type II collagen dissolved in 0.15 *M* phosphate-buffered saline (PBS), pH 7.6 (20 μg/ml). The plates were washed with PBS containing 0.05% Tween-20. The remaining protein-binding sites were blocked by the addition of coating buffer containing 0.5% bovinine serum albumin (BSA); incubation was continued at room temperature for 1 hr. After removal of the PBS–Tween, a 200-μl aliquot of diluted sera (PBS–0.05% Tween–0.5% BSA) was added and the plates were incubated overnight at 4°C. The unbound material was removed by washing the well with PBS–Tween, and 100 μl of peroxidase-conjugated rabbit anti-rat IgG (Cappel Laboratories, Cocharanville, PA) was added at a 1:1000 dilution and incubated for 90 min. After a final wash, 100 μl of orthophenylenediamine (OPD) substrate (40 mg OPD in 100 ml phosphate–citrate buffer, pH 5.0 and 40 μl of 30% H_2O_2) was added to each well. Colorimetric reactions of duplicate samples were read 1 hr later at 490 nm by using a two-wavelength microplate photometer (Model MTP-22, Corona Electric Co., Ltd., Katsuta, Japan). Antibody titers (units/ml) were determined by comparison with an anti-type II collagen antibody standard of 200 units/ml (sera obtained from collagen arthritic rats on Day 50 after immunization) that was included with each assay.

Measurement of skin reaction to type II collagen. Delayed-type hypersensitivity (DTH) skin testing was performed after a slight modification of those described by Griffiths *et al.* (25) and Cremer *et al.* (26). Type II collagen (50 μg) dissolved in 0.05 ml of PBS was injected intradermally in the rat's left ear. The opposite ear was injected with an equal volume of PBS and served as the control. DTH was measured as the change in ear thickness in millimeters (mm) 48 hr after challenge, using an engineer's micrometer, and was expressed as the difference in thickness between the collagen- and PBS-injected ears. Arthus-type reaction was read 3 hr after challenge, according to the method of Mochizuki *et al.* (27) with modification to type II collagen.

Preparation of myelin basic protein (MBP). MBP was prepared from guinea pig spinal cords according to the procedure described by Deibler *et al.* (28).

Induction of experimental allergic encephalomyelitis (EAE). Lewis rats were immunized with an emulsion containing equal volumes of guinea pig MBP (0.2 mg/ml) in saline and complete Freund's adjuvant (FCA) supplemented with 5 mg/ml of *Mycobacterium tuberculosis* H37Ra (Difco Laboratories). Each animal received a total of 0.1 ml of emulsion given equally to the hind footpads.

Clinical evaluation of EAE. Rats were observed daily for EAE and clinical signs of EAE were graded as follows: 0, normal; 1, flaccid tail; 2, hind leg weakness; 3, hind leg paralysis; 4, severe quardriplegia with incontinence. One rat that died during the experiment was assigned a grade of 4.

RESULTS

Dose–Response Studies of FK506 on the Development of Collagen Arthritis

Lewis rats were immunized id with type II collagen emulsified with ICFA, and

given FK506 or placebo fo
matory polyarthritis was i
incidence occurred betwe
examined and the results o
in doses of 0.1 mg/kg or m
mg/kg or more completely

Body weight loss was ol
rats treated with 1.0 mg/kg
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Serum antibody levels t
Even at 0.32 mg/kg, suppr
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Skin responses to type II
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Time Studies of FK506 Tr

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results are given in Table 3.
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5-day course was nearly
FK506 treatment was start
ally suppressed, but its eff
that in the placebo group.

EFFECT OF FK

Agents	D (m
Placebo	
FK506	0
	0
	1
	3

[a] Rats were immunized with t
days. These parameters were ex
[b] Expressed as the difference b
[c] Significantly different from p
[d] Same as footnote [c], $P < 0.00$

Anti-collage antibody was m a-
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s added and the plates were in-
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4.

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n emulsified with ICFA, and

given FK506 or placebo for 12 days, starting on the days of immunization. Inflammatory polyarthritis was induced in all rats treated with placebo, and the peak incidence occurred between 11 and 14 days after immunization. Paw edema was examined and the results on Day 14 are shown in Table 1. Treatment with FK506 in doses of 0.1 mg/kg or more significantly diminished rat paw volume, and 0.32 mg/kg or more completely inhibited arthritis.

Body weight loss was observed in rats treated with 3.2 mg/kg of FK506, or in rat treated with 1.0 mg/kg. This decrease in weight reflected a state of anorexia th... was apparent during the treatment (data not shown). Further toxicological studies are ongoing in our laboratory.

Serum antibody levels to type II collagen were measured by ELISA (Table 2). Even at 0.32 mg/kg, suppression of antibody levels was observed, and complete suppression was obtained at 1 mg/kg or more.

Skin responses to type II collagen were also performed on day 14 after immunization (Table 2). Ear swelling at 3 hr was considered to be arthus reaction rather than DTH reaction. The arthus reaction was inhibited at 0.1 mg/kg or more. DTH response 48 hr after challenge was similarly suppressed. These findings indicate th FK506 suppresses the development of arthritis by decreasing antibody levels and inhibiting arthus reaction and DTH response.

Time Studies of FK506 Treatment on Collagen Arthritis

In this experiment, FK506 was given only during the induction phase of immunity (0–4 days) or only during the immediate preclinical phase of arthritis (7–11 days), to examine time dependency in FK506-induced immunosuppression. The results are given in Table 3. Arthritis was suppressed only if FK506 treatment was st...ted on the day of type II collagen immunization. The results showed that a 5 ...y course was nearly as effective as a 12-day course. In contrast, when FK506 treatment was started on Day 7 after immunization, arthritis was marginally suppressed, but its effect on paw edema was not significantly different from that in the placebo group.

TABLE 1
EFFECT OF FK506 ON TYPE II COLLAGEN-INDUCED ARTHRITIS[a]

Agents	Dose (mg/kg)	Incidence of arthritis	Increment of paw edema (ml)[b]
Placebo	—	10/10 (100%)	1.06 ± 0.03
FK506	0.1	10/10 (100%)	0.53 ± 0.09[c]
	0.32	0/10 (0%)	0.13 ± 0.02[d]
	1	0/10 (0%)	0.09 ± 0.05[d]
	3.2	0/10 (0%)	0.10 ± 0.07[d]

[a] Rats were immunized with type II collagen on Day 0 and treated with FK506 or placebo for 12 days. These parameters were examined on Day 14, when the peak incidence of the disease occurred.
[b] Expressed as the difference between the paw volume on Day 0 and that on Day 14 (mean ± SE).
[c] Significantly different from placebo group, $P < 0.01$.
[d] ...me as footnote [c], $P < 0.001$.

TABLE 2
IMMUNOLOGICAL RESPONSE TO TYPE II COLLAGEN OF FK506-TREATED RATS[a]

Agents	Dose (mg/kg)	Antibody to type II[b] collagen (unit/ml)	Skin response to type II collagen ($\times 10^{-2}$ mm)[c] Arthus	DTH
Placebo	—	175.9 ± 29.3	48.7 ± 1.7	25.9 ± 2.7
FK506	0.1	114.5 ± 38.7	40.6 ± 2.9[d]	16.3 ± 3.5[d]
	0.32	24.4 ± 9.8[e]	21.3 ± 4.5[e]	4.1 ± 1.4[e]
	1	0.4 ± 0.2[e]	8.2 ± 1.4[e]	1.2 ± 0.3[e]
	3.2	0.4 ± 0.2[e]	8.3 ± 1.4[e]	0.8 ± 0.4[e]

[a] Rats were treated as described in Table 1.
[b] Blood samples were taken on Day 14, measured by ELISA, and antibody levels calculated as 200 units/ml on Day 50 after immunization (mean ± SE).
[c] Skin tests were carried out on Day 14 by injecting type II collagen or PBS intracutaneously in rats ears. The responses were read after 3 hr (arthus) and 24 hr (DTH), and expressed as the difference between the ear thickness after injection with type II collagen and that after injection with PBS (mean ± SE).
[d] Significantly different from placebo group, $P < 0.05$.
[e] Same as footnote [d], $P < 0.001$.

Kinetics of FK506-Induced Complete Suppression of Collagen Arthritis

As described above, FK506 in dose of 3.2 mg/kg completely suppressed collagen-induced arthritis, and the kinetics of its effectiveness was examined for 50 days after immunization (Table 4). Even after the cessation of treatment, no arthritic changes were detected.

Immunological responses are shown in Table 5. Antibody levels to type II collagen continuously increased during the experiment in spite of remission of paw edema in the placebo group. In this group, arthus reaction increased until Day 22 and gradually decreased thereafter. DTH reaction in these animals peaked on Day 14 and remained at that level throughout the course of the experiment.

TABLE 3
TIME DEPENDENCE OF EFFECT OF FK506 ON SUPPRESSION OF COLLAGEN ARTHRITIS[a]

Time given (days after immunization)		Incidence of arthritis	Increment of paw edema (ml)
0–11	Placebo	5/5	0.85 ± 0.08
	FK506 (3.2 mg/kg)	0/5 (0%)	0.06 ± 0.01[b]
0–4	Placebo	5/5	0.89 ± 0.17
	FK506 (3.2 mg/kg)	0/5 (0%)	0.04 ± 0.02[b]
7–11	Placebo	5/5	0.84 ± 0.1[b]
	FK506 (3.2 mg/kg)	4/5 (80%)	0.56 ± 0.1[b]

[a] Treatment of rats and parameters are identical to those described in Table 1.
[b] Significantly different from placebo group, $P < 0.001$.

KINETICS OF FK506-INDUCED S

Agents	Dose (mg/kg)	7
Placebo	—	0.01 ± (0/5)
FK506	3.2	−0.05 ± (0/5)

[a] Rats were treated as described in
[b] Increment of paw volume was e represent the incidence of arthritis.
[c] Significantly different from placeb
[d] Same as footnote [c], $P < 0.001$.

FK506 suppressed both hum throughout the experiment foll

Rechallenge Studies of the FK

The specificity of the FK5 was investigated by rechallen collagen with FIA or myelin ba on Day 50 after the primary im immunized, FK506-protected of arthritis after reimmunizati seen on Day 14 after immuniz

KINETICS OF IMMUNOLOGICAL RESP

Days after immunization	Treatment
7	Placebo
	FK506 (3.2 mg/kg)
14	Placebo
	FK506 (3.2 mg/kg)
22	Placebo
	FK506 (3.2 mg/kg)
50	Placebo
	FK506 (3.2 mg/kg)

[a] Rats were treated as described in
[b] These parameters examined as de
[c] Significantly different from placeb
[d] Same as footnote [c], $P < 0.001$.

TABLE 4

KINETICS OF FK506-INDUCED SUPPRESSION ON TYPE II COLLAGEN-INDUCED ARTHRITIS[a]

Agents	Dose (mg/kg)	Days after immunization 7	14	22	50
Placebo	—	0.01 ± 0.01[b] (0/5)	0.98 ± 0.04 (5/5)	0.57 ± 0.10 (5/5)	0.48 ± 0.03 (5/5)
FK506	3.2	0.05 ± 0.01[c] (0/5)	0.02 ± 0.01[d] (0/5)	0.04 ± 0.01[d] (0/5)	0.09 ± 0.01[d] (0/5)

[a] Rats were treated as described in Table 1.

[b] Increment of paw volume was expressed as the mean ± SE (ml). The values in parentheses represent the incidence of arthritis.

[c] Significantly different from placebo group, $P < 0.01$

[d] Same as footnote[c], $P < 0.001$.

FK506 suppressed both humoral and cellular immunity to type II collagen throughout the experiment following the discontinuation of drug treatment.

Rechallenge Studies of the FK506-Protected Rats

The specificity of the FK506-induced suppression of immunologic response was investigated by rechallenging the FK506-protected rats with either type II collagen with FIA or myelin basic protein (MBP) with FCA-supplemented H37Ra on Day 50 after the primary immunization. As shown in Table 6, type II collagen-immunized, FK506-protected rats, except one rat, did not show the development of arthritis after reimmunization with type II collagen (>30 days), but EAE was seen on Day 14 after immunization with MBP. The onset of the latter symptom

TABLE 5

KINETICS OF IMMUNOLOGICAL RESPONSE TO TYPE II COLLAGEN OF NONARTHRITIC RATS TREATED WITH FK506[a]

Days after immunization	Treatment	Antibody to type II[b] collagen (units/ml)	Skin response to type II collagen ($\times 10^{-2}$ mm)[b] Arthus	DTH
7	Placebo	0 ± 0	0.2 ± 1.2	0.2 ± 0.9
	FK506 (3.2 mg/kg)	0 ± 0	0.8 ± 0.6	0.2 ± 0.9
14	Placebo	63.8 ± 0	33.2 ± 1.6	22.4 ± 4.1
	FK506 (3.2 mg/kg)	0 ± 0	8.4 ± 2.2[d]	2.0 ± 0.8[c]
22	Placebo	73.0 ± 0	42.4 ± 1.4	22.4 ± 1.9
	FK506 (3.2 mg/kg)	0 ± 0	2.6 ± 0.7[d]	1.0 ± 0.5[d]
50	Placebo	376.8 ± 25.4	18.2 ± 4.3	22.0 ± 3.3
	FK506 (3.2 mg/kg)	0 ± 0	1.4 ± 2.0[d]	2.0 ± 0.8[d]

[a] Rats were treated as described in Table 1.

[b] These parameters examined as described in Table 2.

[c] Significantly different from placebo group, $P < 0.01$.

[d] Same as footnote[c], $P < 0.001$.

TABLE 6
IMMUNOLOGICAL TOLERANCE INDUCED BY FK506 TREATMENT

Treatment	Arthritis		EAE	
	Incidence	Day of onset	Incidence	Day of onset
Nontreated[a]	10/10	10, 10, 10, 10, 11, 11, 11, 11, 12, 12	10/10	11, 11, 12, 12, 12, 13, 14, 14, 14, 14
FK506 treated Immunized with type II collagen[b]	1/10	30, >30, >30, >30, >30, >30, >30, >30, >30, >30	10/10	11, 12, 12, 12, 13, 13, 14, 14, 14, 14

[a] Nontreated, naive rats were immunized primarily with either type II collagen or MBP.
[b] Rats were treated as described in Table 1. On Day 50 after immunization, secondary immunization was performed with either type II collagen or MBP in each rat treated with FK506 in a dose of 3.2 mg/kg.

coincided with its appearance in the once-immunized naive rats. Thus, the prevention of arthritogenesis produced by a short course of FK506 treatment might be explained as specific immunologic unresponsiveness, which can be defined as the situation where an individual displays a functional nonreactivity to certain antigen while preserving reactivity to others.

DISCUSSION

Our findings show that FK506 suppresses arthritis in type II collagen-immunized rats. They also demonstrate the suppression of anti-type II collagen antibody formation and skin responses to type II collagen (arthus and DTH). Furthermore, FK506-treated rats develop specific unresponsiveness toward type II collagen-induced arthritis.

This suppressive capacity against collagen arthritis was mediated by immunosuppression to type II collagen, not by anti-inflammatory aspects, because FK506 did not reduce the paw edema in acute inflammatory models (data not shown). FK506 is very effective in suppressing arthritis when given in the afferent limbs of the immune responses, but is only marginally effective when given in the efferent limbs. The time dependency of its immunosuppressive effect suggests that it interferes at an early stage of antigenic triggering of immunocompetent cells. A similar time dependency has been reported for cyclosporine (CsA) (15). Kaibara *et al.* (15) demonstrated that CsA treatment only during the induction phase of immunity was successful, whereas the disease worsened, when CsA treatment was started only during the immediate preclinical phase of arthritis. However, FK506 did not exacerbate collagen arthritis when started late. The reason is unclear, but a possible explanation may be obtained when the generation and/or inhibition of suppressor T cells is studied.

It is of interest to note that FK506 is capable of inducing immunologic unresponsiveness in collagen arthritis model. Type II collagen-immunized, FK506-protected rats did not develop arthritis at the same time as naive rats. Only one animal showed the symptoms of arthritis on Day 30 after reimmunization with

type II collagen, and the other reimmunization. However, th with MBP. These results indic FK506 is antigen specific. All thritis. Ochiai *et al.* have sh c: diac allografts was mediate (21).

In conclusion, the results o tively suppresses collagen arth responsiveness to type II coll at the biochemical level is, so l of further investigation.

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e authors express their apprecia tan. and Mrs. Miyuki Baba for her

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type II collagen, and the other animals did not develop arthritis even 30 days after reimmunization. However, they developed EAE in responses to reimmunization with MBP. These results indicate that immunologic unresponsiveness induced by FK506 is antigen specific. Although its mechanism is not known in collagen arthritis, Ochiai *et al.* have showed that FK506-induced unresponsiveness in rat cardiac allografts was mediated by spleen cells by using a cell transfer technique (21).

In conclusion, the results of the present experiments show that FK506 effectively suppresses collagen arthritis, and, moreover, can induce immunological unresponsiveness to type II collagen. Although the mechanism of action of FK506 at the biochemical level is, so far, unknown, these data emphasize the importance of further investigation.

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MISAKO HIRA

*Kidney Disease Section, Natio
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Conversion to Tacrolimus in Cyclosporin A Treated Patients With Gum Hyperplasia

M. Kohnle, P. Lütkes, O. Witzke, T. Philipp, and U. Heemann

KIDNEY transplantation is an established method for the treatment of chronic renal failure. With modern immunosuppressive protocols, graft survival rates after 1 year reached more than 90% in most centers. Therefore, physicians are dealing more and more with problems of long-term immunosuppression.

One complication of Cyclosporin A treatment is gingival hyperplasia in children and adults.[1] The severity of gingival hyperplasia is related to the duration of treatment.[1] Gingival hyperplasia and concomitant gingival bleeding may increase the incidence of oral infections, resulting in a severe reduction of life quality. In some cases surgical gingivectomy is needed.[2] The presence of calcium channel blockers may aggravate this effect.[3] Ultrastructural studies revealed an increased production of amorphous ground substance by fibroblasts,[4] but the exact mechanism of gingival hyperplasia is still unknown.

Tacrolimus has proven to be effective in renal transplantation. In patients treated with tacrolimus, gingival hyperplasia has not been observed. We therefore investigated in a prospective study the effects of a conversion to tacrolimus in recipients of a renal allograft with stable graft function and cyclosporine A induced gingival hyperplasia.

PATIENTS AND METHODS

Recipients of a renal graft receiving a cyclosporin A based immunosuppression with stable graft function (defined as serum creatinine <2.5 mg/dL over the last 90 days before study entry) who suffered from severe gingival hyperplasia were included into the study. Gingival hyperplasia was assessed by an index system defined according to Seymour et al.[5] Briefly, the horizontal component of the hyperplasia index measures the degree of gingival thickening on both the labial and the lingual aspects in a labio-lingual direction for each gingival unit. This was graded from 0 (normal width of free gingival margin) to 2 (thickening >2 mm). The vertical component of the index measures the degree of gingival enlargement in an apico-coronal direction (ie, vertical) for a gingival unit and was graded from 0 (no gingival hyperplasia) to 3 (marked hyperplasia greater than 1/2 of crown length). The patients' gingival condition was assessed using the papilla bleeding index of Saxer and Mühlemann.[6] All measurements were performed by a dentist and taken from the crest of the gingival margin at 6 points around each tooth. All patients received instructions to optimize oral hygiene. Patients were evaluated at study entry and 3 months thereafter.

From the 28 patients included into the study, 15 patients were converted from cyclosporin A to tacrolimus (= tacrolimus group). Patients converted to tacrolimus were adjusted to a tacrolimus trough level of 4 to 8 ng/mL. A control group of 13 patients remained on their initial immunosuppressive regimen. Due to the potential of tacrolimus to increase blood glucose levels, no patient with pathologic glucose tolerance was included into the study. Gingival status and graft function, including drug levels and dose, were determined regularly. All values are given as mean ± standard error of mean.

RESULTS

At study entry, cyclosporin A trough levels were 120 ± 1.4 ng/mL in the tacrolimus group and 142 ± 2.4 ng/mL in controls. After 3 months, trough level was 5.8 ± 0.13 ng/mL in tacrolimus treated patients (Cyclosporine level in controls, 130 ± 1.5 ng/mL).

While hyperplasia score improved from 1.57 ± 0.05 at study entry to 1.30 ± 0.05 after 3 months in the tacrolimus group it worsened in controls (1.20 ± 0.05 at study entry vs 1.40 ± 0.07 after 3 months). The bleeding index dropped in both groups (tacrolimus, from 1.67 ± 0.08 to 1.0 ± 0.09; controls, from 1.34 ± 0.07 to 0.87 ± 0.06). Ten out of 15 patients in the tacrolimus group reported a reduction of pain in contrast to none in controls during the first month after conversion.

There was a slight improvement of renal function in the tacrolimus group, as serum creatinine dropped from 1.9 ± 0.07 mg/dL at study entry to 1.7 ± 0.06 mg/dL after 3 months. By contrast, creatinine increased from 1.7 ± 0.05 at study entry to 1.8 ± 0.05 mg/dL 3 months thereafter in controls.

One patient had to be reconverted from tacrolimus to cyclosporin A due to severe itching. Rejection periods were observed neither in the tacrolimus group nor in controls.

DISCUSSION

In patients with stable graft function, life quality of the patient may be limited by the side effects of immunosuppression. Cyclosporin A is known to cause gingival hyperplasia in a significant proportion of patients. The incidence

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of hyperplasia does not differ significantly between solution and capsules indicating a systemic rather than a local effect.[7] Ultrastructural studies revealed increased amorphous ground substances with a higher level of glykosaminoglykanes. However, the exact mechanism remains unknown.

Frequently, gingival hyperplasia is so severe that life quality is impaired. Hyperplasia may be aggravated by concomitant use of calcium channel blockers. Optimal oral hygiene alone was not sufficient to reduce hyperplasia, as demonstrated by the reduced bleeding index in both groups without an improvement in the hyperplasia index in patients remaining on cyclosporin A.

As tacrolimus is known to be effective in transplant recipients over the long term, a conversion to tacrolimus may be considered in patients with severe gum hyperplasia secondary to cyclosporin A. In addition to pain reduction, objective measurements revealed a marked decrease of bleeding and hyperplasia as early as 3 months after conversion. Following conversion, we observed no rejections. However, a longer follow-up is needed to draw reliable conclusions. Interestingly, creatinine dropped after conversion, an indication for a lower nephrotoxicity of tacrolimus.

In conclusion, a switch to tacrolimus is safe and beneficial in patients with gingival hyperplasia.

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efficacy of FAST as a means of obtaining joint remission needs to be reconsidered and studied further.

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USE OF NABUMETONE (NAB) TO TREAT JUVENILE RHEUMATOID ARTHRITIS (JRA) IN CHILDREN INTOLERANT OF OTHER NON-STEROIDAL ANTI - INFLAMMATORY DRUGS (NSAIDs). BE Ostrov; Penn State Univ, Hershey, PA

Recent studies suggest that children treated chronically with NSAIDs, such as in JRA, may have up to 75% incidence of endoscopically proven NSAID gastropathy (J Pediatr 1993, 122:647) and symptoms of gastrointestinal intolerance are common (AJDC 1993 147:299). NAB is an NSAID designed as a pro-drug which must be transformed in the liver to its active moiety which has anti-inflammatory properties. NAB has been shown, in adults, to cause significantly fewer signs and symptoms of gastrointestinal toxicity compared to other NSAID (Arthritis Rheum 1995, 38:5). No studies of pediatric use of NAB have been published. However, due to its gastric-sparing properties, after parental consent, NAB was offered to and used by 15 JRA patients with prior NSAID intolerance.

All patients met ACR criteria for the diagnosis of JRA. The children ranged in age from 5 to 16 years (mean 10.7 years). Diagnoses were: polyarticular JRA in 8 patients, pauciarticular JRA in 5 cases and systemic JRA in 2 children. 7/15 patients were stable on methotrexate, and/or sulfasalazine and 2 were taking stable, low dose prednisone. All patients had been intolerant of one or more prior NSAIDs leading to discontinuation of these drugs. All patients had recurrent abdominal pain and/or nausea, two had gastrointestinal bleeding and one child had had a documented peptic ulcer. NAB was dosed for 5 to 27 months (mean 11.7 months) at 10 to 30 mg/kg/d up to 2000 mg/day (typical maximum adult dosage). Clinically NAB was well tolerated in 14/15 patients with complaints of slight nausea in one child. Laboratory monitoring revealed no changes from baseline in 13/15 patients. Two children developed asymptomatic proteinuria (450 and 1100 mg/d). Disease control was improved in 11/15 patients and stable in 4/15 when compared to therapy with prior NSAID. Second line therapy was added or increased in 4 children due to persistent arthritis. Fever was not controlled by NAB in the two children with systemic JRA. NAB was discontinued in the one child with 1100 mg/d proteinuria but was not stopped in the child with 450 mg/d proteinuria because of severe gastrointestinal toxicity with prior NSAID use.

NAB appears to be a well-tolerated NSAID in children with JRA with little toxicity noted in this open trial. Prospective, controlled toxicity and efficacy studies need to be performed to further assess the usefulness and safety of NAB in the pediatric population.

DISCLOSURES: NONE

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FK506 IN SEVERE TREATMENT RESISTANT JUVENILE RHEUMATOID ARTHRITIS (JRA). Anita Goel, Aldo Vincent Lordino, Jr., University of Pittsburgh and Children's Hospital of Pittsburgh, Pittsburgh, PA 15213

Tacrolimus (FK506) is an immunosuppressive drug more potent than but structurally unrelated to cyclosporin which also inhibits T-cell activation and decreases the production of interleukin-2 and other cytokines thought to play a central role in the inflammatory processes of JRA.

The purpose of our study was to evaluate the effect of FK506 on severe refractory JRA. 4 Caucasian female patients with JRA onset at ages 2 to 9 years, and a mean disease duration of 6.4 years were treated with FK506. All met ACR criteria for JRA (3 systemic onset, 1 pauciarticular onset) and had a subsequent severe polyarticular course.

Prior to initiating FK506 all patients had been treated concomitantly with NSAIDs, corticosteroids, subcutaneous methotrexate (up to 1mg/kg/wk), intravenous immunoglobulin and plaquenil. 3 of the 4 patients were also on sulfasalazine. NSAIDs and methotrexate were discontinued prior to initiating FK506, all other medications were continued at a stable dose with no new additions. FK506 was maintained at therapeutic levels of 10 to 20ng/ml for a mean treatment duration of 10.8 months.

All patients showed a significant improvement in disease activity as reflected by a reduction in corticosteroid requirement, hospital admission rate and joint count, as well as an improvement in laboratory markers of disease activity (ESR, hematocrit, platelet count, serum albumin and interleukin-2 receptors) and physician assessment of overall disease activity. After one year of treatment with FK506, the pauciarticular onset patient developed a polyarticular flare at a low therapeutic FK506 level. No major adverse effects were noted with treatment. In conclusion, FK506 may be beneficial in patients with severe refractory JRA.

most children with JRA who respond to treatment with a higher likelihood of response to treatment.

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MAGNETIC RESONANCE IMAGING IN THE Tae Hwan Kim, Kwan Pyo Hong, Jae Bum Jun, Sun Hyun Yoo, Seong Yoon Kim, Rheumatism Center, I Diagnostic Radiology, Hanyang University Hospital, S

It is generally accepted that magnetic resonance im information regarding the disease process in early subchondral bone and periarticular bone marrow. W pain but normal or suspicious plain radiographic ch subchondral bone and periarticular bone marrow.

Forty two sacroiliac joints(SI) in 21 patients were e the radiologic criteria for sacroiliitis of Kellgren, wer II, 26 SI; grade III, 2 SI. We compared spin echo(contrast T1 image), MPGR. The early image of post enhancement; the late image, 16 minutes.

Periarticular fat accumulation, osteitis, and pannu prominant than on the sacral side. As the radiogra dominant.The periarticular fat accumulation is domi portion of SI. The amount of periarticular fat accur disease. In post contrast image pannus was well visu better than the early image, but was of no statistic grade I had nodular pattern but became more linear than MPGR in detection of periarticular fat accumul MPGR was superior to spin echo in detecting subchon

It appears that MRI is superior in detecting the abn plain film. There was no comparison between spin ec detection of sacroiliitis, spin echo can detect periarti MPGR and is superior in diagnosing the condition. I better, while the delayed images of post contrast are g

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JUVENILE RHEUMATOID ARTHRITIS IN R THE INCIDENCE DECREASING? L.S. Pe O'Fallon, S.E. Gabriel, Mayo Clinic, Rochester,

The goal of this study was to examine trends rheumatoid arthritis (JRA) in Rochester, Minnes

The diagnostic retrieval system of the Roch utilized to screen medical records of all Rochest JRA (utilizing ≥ 20 diagnostic categories) f Rheumatism Association 1977 revised criteria) previously identified cohort from 1960-1979 combined resulting in an incidence cohort spann Of the 1240 medical records screened, we ider diagnosed between 1960 and 1993. The averag years) for a total of 826 person-years of obse diagnosis was observed with peaks between 0-4 of patients had pauciarticular onset, 17% had onset. Progression of pauciarticular to polyartic overall age- and sex-adjusted incidence rate wa (4.8). The incidence rate per 100,000 popula periods 1960-1969, 1970-1979, and 1980-1993, moving average, used to display time trends in incidence peaks in 1967, 1975, and 1987.

An overall decrease in the incidence rate over the pauciarticular- and systemic-onset subtyp cyclical pattern, suggest that environmental facto

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A Case of Pyoderma gangrenosum Stabilized with Lymecycline, Topical Benzoyl Peroxide and Treated by Autograft

Pyoderma gangrenosum is a chronic inflammatory ulcerative skin disease of unknown etiology, often associated with various systemic disorders such as inflammatory bowel disease, rheumatoid arthritis, chronic active hepatitis, diabetes mellitus and hematologic malignancies. The ulcers are characterized by their undermined violaceous borders. The disease remains a therapeutic challenge. Corticosteroids are the mainstay of therapy; however, side effects from this treatment and recalcitrant pyoderma gangrenosum require therapeutic alternatives. We report the case of a large subacute pyoderma gangrenosum stabilized with lymecycline, topical benzoyl peroxide and successfully treated by an autograft. This observation supports the opinion that the risk of pathergy of a graft can be avoided by the stabilization of the disease.

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METHOTREXATE AND AZATHIOPRINE IN RA: RADIOLOGIC PROGRESSION AFTER 4 YEARS, A PROSPECTIVE STUDY. P Kerstens, A Boerbooms, M Jeurissen, R de Graaf, J Mulder, L vd Putte. Depts of rheumatology and medical statistics, University Hospital Nijmegen, PO BOX 9101, 6500 HB Nijmegen, the Netherlands.

Objective: To study whether the beneficial effect of MTX on radiologic progression after 1 year as compared with AZA, which we recently reported, was sustained after 2 and 4 years (yrs).
Methods: Open extension of follow-up of our 48-weeks double-blind comparative study of MTX and AZA to 4 yrs. Follow-up included radiographs of hands and feet at 2 and 4 yrs. Changes in radiologic scores were studied according to both a same drug analysis and an intention-to-treat analysis.
Results: After 4 years 18 patients (pts) (58%) from the MTX group continued the initial study drug, and 7 pts (21%) in the AZA group. More patients (n=21) switched from AZA to MTX, than vice versa (n=5). In the same-drug analysis no significant differences in increment in radiologic scores was found. Results of intention-to-treat analysis are shown in the Table.

Erosion score(E) and Total score (T), changes from baseline (95%CI).

	AZA			MTX		
yr	1 (n=33)	2 (n=31)	4 (n=31)	1 (n=28)	2 (n=27)	4 (n=23)
E	5.3 (3.4,7.2)	6.5 (4.3,8.8)	10.8 (7.5,14.1)	1.8 (0.7,2.8)*	3.5 (1.6,5.4)#	6.8 (3.3,10.2)@
T	8.6 (6.2,11.0)	11.9 (8.5,15.3)	20.5 (15.4,25.7)	4.7 (2.0,7.5)@	7.7 (3.9,11.4)$	14.2 (7.5,20.9)

Comparison MTX vs AZA: * P = 0.002; # P = 0.05; P = 0.09; @ P = 0.03.

Conclusions: Drug survival after 4 years was better for MTX than for AZA. In an intention-to-treat analysis the beneficial effect of MTX on radiologic progression after 1 year compared with AZA was sustained after 2 years and probably also after 4 yrs. Differences tend to level off, probably due to the greater number of switches from AZA to MTX than vice versa.

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THE USE OF FK506 (TACROLIMUS) IN THE TREATMENT OF RESISTANT RHEUMATOID ARTHRITIS. Richard B. Gremillion, James Posever, Nisha Manek, John P. West, Ronald F. van Vollenhoven. Division of Rheumatology, Stanford University Medical Center, Stanford, CA 94305

Introduction: FK506 is a potent antiinflammatory and novel immunosuppressive agent. It inhibits cell-mediated immunity in vivo and is replacing cyclosporin (CYA) in preventing transplant rejection secondary to improved graft survival. It has been used successfully to rescue transplant patients intolerant of CYA and those showing signs of ongoing rejection. Renal toxicity with FK506 may be less given the fact that FK506 doses, in transplant studies comparing FK506 with CYA, are >2-fold higher than those generally used in rheumatic disease.

Purpose: An open label, pilot study to assess whether FK506 is safe and potentially useful in patients with severe, refractory RA who have failed or shown only modest response to traditional DMARD therapy.

Patients and Methods: 12 pts (2 men, 10 women) with RA, mean age 49, and disease duration 9.4 years were identified with the following averaged characteristics: erythrocyte sedimentation rate (ESR) 48.5 ± 10.7, morning stiffness (MS) 214 ± 61.2 minutes, Swollen joint count (SJC) 17.7 ± 2.5 and tender joint count (TJC) 26.4 ± 4.18. 1 pt had failed six, 10 five, and 1 four DMARDs (i.e hydroxychloroquine, sulfasalazine, methotrexate, azathioprine, CYA & penicillamine). 9/12 pts were on prednisone (ave 12mg/d). 8/12 pts were intolerant of CYA. Four pts continued to take HCQ and three MTX. All pts had nl creatininers at baseline. Each pt was begun on 1mg FK506 bid. Dosage was increased to 2mg bid at 1 month and 3mg bid at 2 months. Monthly monitoring was performed.

Results: Therapy with FK506 was discontinued in 5 patients. Four were intolerant of FK506 and one (ACR 50 responder at 4 weeks) discontinued therapy after an unrelated episode of antacid-responsive chest pain. Pt 1- nausea at 5 wks, pt 2- nausea & headache at 6 wks, pt 3- nausea & diarrhea at 9 wks & pt 4- worsening carpel tunnel pain and arthralgia. 7/12 (58.3%) continue on therapy without side effects (4 of which showed prior CYA related creatinine elevation). No rise in serum creatinine has been observed in any of our study pts. 7 pts have completed 16 wks of therapy and show statistically significant improvement in TJC (from 17.7 ± 4.2 to 7 ± 3.2, p=.021). 5 pts have completed 24 wks of therapy and show statistically significant improvement in ESR (from 48.5 ± 10.7 to 12.8 ± 4.4, p=.047), HAQ (from 1.625 ± .27 to .893 ± .23, p=.002), SJC (from 17.7 ± 2.5 to 3.8 ± 1.7 p=.006), TJC (from 26.4 ± 4.2 to 13 ± 4.2, p=.049), Pt Global (from 57.4 ± 8.7 to 20 ± 5.9, p=.022), MD Global (from 61.7 ± 8.6 to 16.2 ± 6.5, p=.006). At 16 wks 6/7 pts are ACR 20 responders & 3/7 are ACR 50 responders. All 5 pts completing 24 wks are ACR 50 responders.

Conclusions: Although FK506 was not tolerated by nearly half of the patients in this study, the patients who were tolerant showed excellent clinical repsonses. Thus, FK506 shows promise as a rescue agent in the treatment of severe, refractory RA in selected patients.

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CYCLOSPORINE A THERAPY IN RHEUMATOID ARTHRITIS: ONLY STRICT APPLICATION OF THE GUIDELINES FOR SAFE USE CAN PREVENT IRREVERSIBLE RENAL FUNCTION LOSS. B.E.E.M. van den Borne[1], R.B.M. Landewé[2], H.S. Goei The[2], F.C. Breedveld[1], B.A.C. Dijkmans[3]. Department of Rheumatology, Leiden University Hospital[1], Atrium Medical Center Heerlen[2] and Free University Hospital Amsterdam[3], The Netherlands.

Objectives: To investigate whether the increase in serum creatinine observed during cyclosporine A (CsA) - therapy was reversible in a group of patients with advanced rheumatoid arthritis (RA) treated before the current guide-lines for safe use of CsA in RA were developed. These guidelines state that CsA dosages should be decreased when serum creatinine levels increase above 30% of baseline.

Patients and methods: Eighty-three patients with advanced RA who had started low-dose CsA therapy between September 1990 and October 1992 were followed until drug-discontinuation. All 83 patients were investigated in December 1995. The serum creatinine increase level was determined in patients who had discontinued CsA for at least 3 months. Predictors for irreversibility of renal function loss were determined by using multiple regression analysis.

Results: The mean level of serum creatinine gradually increased from 69 ± 14 (mean ± SD) µmol/l at entry to 88 ± 23 µmol/l (28% above baseline) at the moment of drug-discontinuation, and decreased to 80 ± 17 µmol/l (16% above baseline) during follow up of 35 ± 14 months after drug-discontinuation. During CsA therapy, the mean level of serum creatinine had increased to 82 ± 19 µmol/l (26% above baseline) at 6 months and to 87 ± 22 µmol/l (39% above baseline) at 42 months. The mean CsA-dose was 3.1 ± 0.9 mg/kg/day at 6 months and 1.9 ± 0.8 mg/kg/day at 42 months. The absolute number of months that serum creatinine levels were higher than 30% above baseline was an independent predictor for a persistent increase of the serum creatinine after CsA discontinuation (β = 0.506; p<0.001). Patients with ≤ 2 months with serum creatinine levels higher than 30% above baseline had a mean irreversible increase at follow up of 6% (p>0.05), compared to 27% in patients with > 2 months (p<0.0001).

Conclusion: Long term low-dose CsA-administration in patients with advanced RA was associated with a persistent increase in serum creatinine which was partially irreversible after drug-discontinuation. The increase in serum creatinine was reversible in the patients who were treated according to the current guide-lines for safe use of CsA.

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Sandoz and the Leiden University Medical Center.

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DOES LONG-TERM CYCLOSPORINE-A TREATMENT IN RHEUMATOID ARTHRITIS PROTECT AGAINST CANCER ? B.E.E.M. van den Borne[1], R.B.M. Landewé[2], F.C. Breedveld[1], H.J. Bernelot Moens[3], B.A.C. Dijkmans[4]. Departments of Rheumatology, Leiden University Hospital[1], Atrium Medical Center Heerlen[2], Medisch Spectrum Twente[3] and Free University Hospital Amsterdam[4], The Netherlands.

Background: In a retrospective controlled cohort study on the potentially carcinogenic effects of cyclosporin-A (CsA) in patients with rheumatoid arthritis (RA) we have found a relative risk (RR) of CsA-treated patients to contract cancer of 0.41 (95%CI: 0.19-0.89) compared to matched controls. The purpose of this study was to determine whether there is a relationship between the duration of exposure to CsA and the level of risk reduction.

Methods: The incidence of cancer was established in 208 RA-patients treated with CsA in previous clinical trials and of 415 control RA-patients (75 of them were randomized control patients derived from the controlled CsA-trials) treated simultaneously with other DMARDs. Cox proportional hazard-regression analysis was performed to correct for potentially confounding variables.

Cases of cancer per 1000 follow-up years according to treatment duration

	< 1 year	*adjusted RR*	*> 1 year*	*adjusted RR*
CsA-group	9.1	0.64 (0.20-2.11)	3.1	0.16 (0.03-0.76)
Control-group	15.2		17.2	

The results were similar in the analysis of only the randomized patients. In the CsA-group duration of treatment was significantly related to the chance on cancer (adjusted RR: 0.75(0.61-0.92) per extra month of treatment).

Conclusion: The negative association between CsA and the risk on cancer in RA is dependent on the length of exposure to CsA, which strengthens the hypothesis of a protective effect of CsA, compared to other DMARDs.

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THREE-YEAR CHANGES IN RADIOGRAPHIC DAMAGE AND PHYSICAL DISABILITY IN 278 RHEUMATOID ARTHRITIS (RA) PATIENTS RANDOMIZED TO GOLD (GST) OR CYCLOSPORIN (CYA). TK. Kvien, Oslo City Dept. of Rheumatology, Diakonhjemmet Hospital, N-0319 Oslo, Norway, HK Zeidler, Hannover, Germany, P Hannonen, Jyväskylä, Finland, FA Wollheim, Lund, Sweden, P Kurki, Novartis, Basle, Switzerland, and the SIMERA Study Group.

It is recognized that DMARDs have a weak short-term effect on radiographic progression, but data over more than 1-2 years are sparse.

RA patients with active and severe disease of less than 3 years duration (n=278, mean age 48 years, females 70%, mean disease duration 12 months, systemic corticosteroids in 54%) were randomized to CYA (3 mg/kg with dose adjustment according to international guidelines) or GST (50 mg/week, 50 mg/month after 1000 mg total dose). X-rays (read blind for treatment code and for the sequence of the x-rays of a given patient) and HAQ were assessed at baseline, after18 and 36 months.

Number of treatment terminations due to inefficacy or adverse reactions were similar in both groups. The table shows results from the intention to treat population (n=278). Similar results were seen when analysing the completers (CYA n=56, GST n=44) except no difference regarding changes in disability. Radiographic progression in both groups was associated with baseline levels of acute phase reactants, number of swollen joints, damage scores, and rheumatoid factor.

	Baseline		Changes 18-0		p-value	Changes 36-0		p-value
	CYA	GST	CYA	GST		CYA	GST	
Larsen score	14.8	14.1	10.0	10.3	0.42	15.5	13.9	0.52
No. of eroded joints	4.0	4.0	2.6.	2.8	0.50	3.7	3.8	0.61
No. of erosions	7.5	7.5	5.8.	5.3	0.25	10.0	8.4	0.30
HAQ score	1.12	1.05	-0.50	-0.40	0.21	-0.54	-0.45	0.04

In conclusion, damage progressed despite improvement in physical function; the numeric X-ray changes from 18 to 36 months were lower than during the first 18 months of the study; and only approximately 1/3 of the patients completed full 3-year treatment with their original therapy.

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TREATMENT OF ACTIVE RHEUMATOID ARTHRITIS WITH LEFLUNOMIDE IMPROVES FUNCTIONAL ACTIVITIES AND HEALTH RELATED QUALITY OF LIFE (HRQOL) [1]P Tugwell, [2]C Bombardier, [3]V Strand, [4]A Maetzel, [1]G Wells and the Leflunomide RA Investigators Group, [1]Ann Thompson [1]University of Ottawa, Ottawa ON; [2]University of Toronto, Toronto ON; [3]Stanford University, Stanford CA 94305; [4]Hoechst Marion Roussel, Kansas City MO 64134

This 12 month multi-center randomized placebo controlled trial evaluated the safety and efficacy of leflunomide (LEF) 20 mg daily (100 mg x 3 days loading dose) compared to placebo (PL) and methotrexate (MTX) 7.5-15 mg weekly in 482 patients (182 LEF, 118 PL, 182 MTX) with active rheumatoid arthritis (RA). Treatment groups were similar in baseline (BL) demographics, mean disease duration: 6.7 years, prior DMARDs failed: 0.8. Modified Health Assessment Questionnaire (MHAQ) was administered monthly; HAQ, Problem Elicitation Technique (PET) and Medical Outcomes Survey Short Form 36 (SF-36) at BL, 24 and 52 weeks or early treatment exit.

Statistically significant improvement in function and HRQOL were reported with LEF treatment compared to PL: by MHAQ, all scales of HAQ and disability index, weighted top 5 score of PET, 5 of 8 subscores of SF-36 and work productivity. In comparison to MTX, LEF treatment resulted in significant improvements in MHAQ, 5 of 8 scales of HAQ and disability index, weighted top 5 of PET and 2 of 8 subscores of SF-36. (Intent to Treat Population, Baseline to endpoint)

	LEF	PL	MTX	p values
n	166	101	169	
MHAQ: Baseline	0.78	0.89	0.79	* LEF vs PL: ≤0.0001
Mean Change	-0.29 *†	0.09	-0.15	† LEF vs MTX: p ≤0.01
HAQ: Disability Index: BL	1.3	1.3	1.3	* LEF vs PL: ≤0.0001
Mean Change	-0.45 *†	0.0	-0.03	† LEF vs MTX: p ≤ 0.01
PET Weighted Top 5: BL	21.2	22.4	20.4	* LEF vs PL: ≤0.0001
Mean Change	-6.91 *†	-0.66	-3.41	† LEF vs MTX: p ≤ 0.001
SF-36 Physical Component: BL	30.0	28.9	29.7	* LEF vs PL: ≤0.0001
Mean Change	7.6 *†	1.0	4.6	† LEF vs MTX: p ≤ 0.01
Work Productivity: BL	53.3	52.9	51.9	* LEF vs PL: ≤0.01
Mean Change	9.8 *	0.3	7.5	

Disability at baseline improved in both active treatment groups. LEF resulted in statistically greater improvement than MTX in health status measures and functional activities important to the patient.

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This study was supported by Hoechst Marion Roussel. Drs. Bombardier, Maetzel, Strand, Tugwell and Wells are consultants to HMR.

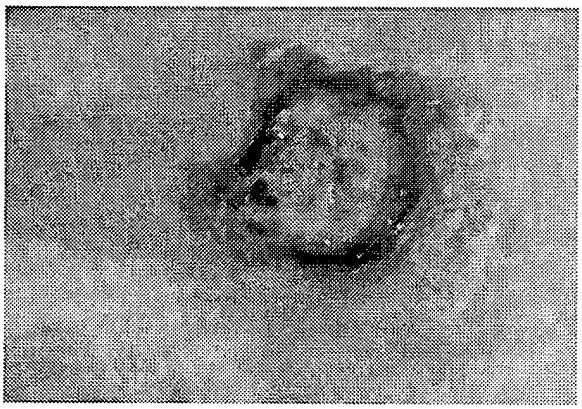
Pyoderma gangrenosum

Pyoderma gangrenosum was first used to describe a "destructive cutaneous disorder, characterised by painful, rapidly enlarging ulcers with undermined bluish and purplish-red margins" in relation to inflammatory bowel disease. Brunstring et al 1930.

Pyoderma gangrenosum is now thought to be of an immunological origin, although the pathenogenisis is not clear. It is often associated with systemic diseases such as rheumatoid arthritis, chronic inflammatory bowel disease, Crohn's disease, Ulcerative colitis, Monoclonal gammopathy and rarely with leukaemia. It may occur however without any reported underlying disease.

Harland & Millard (1993) reported on three patients with venous leg ulcers that deteriorated inexplicably, to be later diagnosed as pyoderma gangrenosum.

As Pyoderma gangrenosum is not caused by bacteria, antibiotics are of no benefit in treating the underlying condition. (they may be needed to control secondary infections.) Skin grafting is also ineffective. (Schwaitzberg et al 1982)



A Classic pyoderma gangrenosum, no eschar, purple undermining border, haemorrhagic blister and a red peripheral border.

Clinical Appearance (after Prystowsky et al 1989)

- Lesions may occur singly or in groups, most commonly on the lower limbs, although they may appear anywhere on the body.
- Have a liquefying centre without eschar formation
- Purple Undermining boggy borders, that may be covered by haemorrhagic blisters
- Peripheral red borders
- Frequently painful

Treatment

The mainstay of treatment of pyoderma is the use of high dose systemic steroids. Response to steroids has been used to confirm diagnosis of pyoderma gangrenosum. Some work has been reported on the use of immunosuppressant drugs such as cyclosporin, with favourable results. (Duguid & Powell 1993)

Woundcare is typically, surgical debridement of the ulcer, then a moist wound healing régime, Foams, hydrogels, hydrocolloids and alginate dressing have all been reported as being used. (Samuel & Williams 1996)

Prognosis

Ko et al (1992) reviewing 14 cases over a 24 year period found healing times of 3 months to 6 years. Some patients experienced recurrence of the ulcer.

Two case studies reported in the Journal of Woundcare had healing times of around 4 months from diagnosis. (Samuel & Williams 1996, and Sutherland 1997)

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oderma gangrenosum | Cavity Wounds | Fungating | Diabetic Foot | Malodour

Bite Wounds | Burns

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Brief Report

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Acute Pyoderma Gangrenosum Does Not Require Surgical Therapy

Yong-Kwang Tay, MD; Marti Friednash, MD; John L. Aeling, MD

Pyoderma gangrenosum is an uncommon ulcerative skin disorder that is often associated with underlying systemic diseases, the most common of which is inflammatory bowel disease. We report an illustrative case of a 36-year-old woman with pyoderma gangrenosum occurring at surgical sites and at sites of trauma. She had associated Crohn disease. Multiple surgical procedures were performed on this patient, without improvement. Pyoderma gangrenosum can mimic a necrotizing soft tissue infection. Early recognition of the characteristic lesion may prevent unnecessary operations and facilitate effective control with appropriate medical therapy.

Arch Fam Med. 1998;7:377-380

REPORT OF A CASE

In April 1994, a 38-year-old white woman noted erythematous, painful plaques of the left palm and the proximal phalanx of the left middle finger (Figure 1). This was thought to be cellulitis, and a regimen of broad-spectrum antibiotics was started. The lesions were subsequently débrided. A similar lesion appeared on the distal phalanx of the same finger. She was transferred to the University of Colorado Health Sciences Center, Denver, for further evaluation.

The patient had similar symptoms of her right third finger in September 1992 after blunt trauma to the same finger. New lesions developed on her right second and fourth fingers despite broad-spectrum antibiotic therapy. Surgical débridement was performed for suspected necrotizing soft tissue infection. Five days later, lesions recurred at the surgical margins despite cultures being negative for pathogens. Further surgical treatment was undertaken, resulting in amputations of the right second, third, and fourth digits (Figure 2). The wound healed uneventfully.

Her medical history was notable for Crohn disease that was diagnosed in 1991 and treated with prednisolone. In March 1992, a rectovaginal fistula developed, requiring a diverting colostomy. Parenteral steroid therapy was discontinued in the fall of 1992. One year later, a regimen of prednisolone and azathioprine was started for a flare of her intestinal disease. Prednisolone was subsequently discontinued. Thus, the patient's lesions corresponded to periods when she was not taking prednisolone.

Social and family histories were noncontributory.

On examination, the patient was afebrile. The left middle finger was swollen and tender with erythematous, dusky plaques on the left palm and the distal phalanx of the left middle finger. An open wound with purple margins was seen of the proximal phalanx at the site of the surgical débridement (Figure 3). Erythematous, violaceous plaques were present on the left thigh and the dorsum of the right foot, which was the site of an intravenous line. Laboratory investigations revealed an elevated white blood cell count of 11.6×10^9/L (11600/μL) with a differential count of 0.80 (80%) polymorphonuclear leukocytes. The erythrocyte sedimentation rate was 92 mm/h. Histological examination of a biopsy specimen taken from the edge of the ulcer on the left middle finger showed a nonspecific, diffuse, neutrophilic dermal infiltrate with scattered chronic inflammatory cells and areas of focal necrosis. Cultures of the biopsy material were negative for acid-fast bacilli, bacteria, and fungi. The results of the following investigations were either normal or negative: blood biochemistry tests, liver function tests, hepatitis B and C serologic tests, complement levels, antinuclear antibody test, and serum and urinary protein electrophoresis.

A diagnosis of the pyoderma gangrenosum associated with Crohn disease was made. Pulse therapy with methylprednisolone sodium succinate, 1 g/d for 3 days, was given. A dramatic response occurred within 12 hours with a reduction of pain and erythema. Her lesions started to heal within a week, and she was discharged with a regimen of prednisone, 60 mg twice a day, and sulfasalazine, 1 g twice a day.

COMMENT

Pyoderma gangrenosum is an uncommon necrotizing and ulcerating skin disease that was first described by Brunsting et al[1] in 1930. The diagnosis is made clinically because no specific histopathologic or immunofluorescent patterns are present.[2] The earliest symptom may be pain in the area, followed by small erythematous papules. The lesions rapidly evolve into tender pustules surrounded by indurated erythematous skin that breaks down to form an ulcer. The rapidity of the development of the lesions and the appearance of the ulcers, with their pus-covered centers and the ragged, undermined, violaceous borders, are hallmarks of the disease and distinguish it from soft tissue infection.[3] Vesicles and bullae may be present,[4] as in our patient. Lesions are most commonly found on the lower extremities[2] but have been reported on the scalp, face, trunk, and arms. Lesions may be single or multiple and may be precipitated by trauma (pathergy), as in our patient. Pathergy is a condition in which the application of a stimulus makes the organism unduly susceptible to a subsequent stimulus of a different kind. The Behçet and the Sweet syndromes also involve this type of pathergy. A history of pathergy is reported in 25% of patients.[5] Ulcers heal with cribriform, atrophic scars.[1] All age groups are involved, with the youngest documented patient being a 3-week-old infant.[6] Peak incidences occur in the third and fourth decades of life for female patients, as in our patient, and in the fifth decade of life for male patients.[3]

Pyoderma gangrenosum is a marker of various systemic diseases (Table 1). It is most often associated with inflammatory bowel disease.[25] It

occurs in ..... to ... of ,atients ..ith ulcerati.e colitis and in 0.8% to 1.5% of patients with Crohn disease.[26] The appearance of pyoderma gangrenosum is nearly always preceded by the inflammatory bowel disease,[2] as in our patient. Exacerbations of skin lesions tend to parallel recurrences of the intestinal inflammation.[27]

Pyoderma gangrenosum has been reported to occur during granulocyte colony-stimulating factor therapy.[7,8] The up-regulation of neutrophilic function and secondary release of cytokines may induce this complication.[28] In 40% of patients with pyoderma gangrenosum, no associated disease can be identified.[2]

The cause of pyoderma gangrenosum remains obscure. Brunsting et al[1] initially called it a pyoderma because it was thought to be a bacterial infection caused by streptococci or staphylococci. The cause is now recognized to be noninfectious, although secondary bacterial colonization may occur. Recent investigations emphasize an altered immune system with impaired cellular immunity and defective function of polymorphonuclear leukocytes.[9]

The histopathologic appearance of pyoderma gangrenosum is not diagnostic (Figure 4). Early lesions show a deep folliculitis with numerous neutrophils surrounding the pilosebaceous unit.[29] Vascular changes may be noted. Later, changes of suppurative granulomatous dermatitis supervene, with collections of histiocytes, macrophages, and giant cells. Massive papillary dermal edema may occur. Finally, lesions regress with marked fibroplasia.[30] Although the histopathologic features are not diagnostic, a skin biopsy is necessary to rule out other causes of acute skin ulcerations, particularly infections and necrotizing vasculitis.

The differential diagnoses for pyoderma gangrenosum include bacterial infection, synergistic gangrene, deep fungal infection, necrotizing vasculitis, bullous erythema multiforme, Sweet syndrome, Behçet syndrome, halogen dermatitis, brown recluse spider bites, amebiasis, purpura fulminans, and factitial ulcer.[3] The diagnosis of pyoderma gangrenosum is based on the clinical appearance of the lesion, its association with systemic disease, the exclusion of other causes of dermatitis, and a poor response to antibiotics.[10] In the absence of associated systemic

disease, the diagnosis is more difficult but is based on the same variables.

The management of pyoderma gangrenosum is directed at the systemic disease, with high-dose corticosteroid therapy and local wound care. Corticosteroid therapy has been the main method of treating pyoderma gangrenosum.[2] Initial doses of prednisone of up to 80 to 120 mg/d may be given, with subsequent tapering of dosages to maintenance levels.[2] Johnson and Lazarus[11] used pulsed therapy of methylprednisolone sodium succinate, 1 g intravenously, in 150 mL of 5% dextrose solution for 5 days to treat refractory pyoderma gangrenosum. They observed a dramatic response with minimal adverse effects. Our patient had a rapid response to pulsed methylprednisolone therapy with a reduction of pain and erythema within 12 hours. Intralesional steroids, in the form of triamcinolone acetonide suspension, have been used to treat early lesions of pyoderma gangrenosum, with good results.[12] Other drugs that have been used to treat pyoderma gangrenosum, either alone or in conjunction with steroids, with variable success include sulfones such as dapsone[13] and sulfasalazine,[3] clofazimine,[14, 15] minocycline hydrochloride,[16] potassium iodide,[17] colchicine,[18] human intravenous immune globulin,[19] and immunosuppressive agents such as azathioprine,[2] cyclophosphamide,[20, 31] chlorambucil,[21] cyclosporine,[22, 23] and tacrolimus.[24, 32] Any of these agents can be used without associated systemic disease. Reported topical agents that have been used successfully include topical cromolyn sodium[28, 31] and topical mechlorethamine hydrochloride.[30] Hyperbaric oxygen also has been used successfully to treat pyoderma gangrenosum.[33, 34] Surgical procedures such as débridement and skin grafting are not recommended during the acute stage of pyoderma gangrenosum, especially in patients who exhibit pathergy, because this could lead to further tissue destruction and progression.[35]

CONCLUSIONS

We have presented a case of pyoderma

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gangrenosum associated with Crohn disease that mimicked a soft tissue infection. Besides inflammatory bowel disease, other important associated systemic diseases include hematologic and rheumatologic conditions. Although acute pyoderma gangrenosum may simulate an infection, the characteristic morphologic appearance of the lesions, the association of systemic disease, cultures that are negative for pathogens, results of a skin biopsy that are consistent with the diagnosis, and a failure to respond to antibiotic therapy should point strongly to a diagnosis of pyoderma gangrenosum. High-dose parenteral corticosteroids are the treatment of choice for acute pyoderma gangrenosum. Often a steroid-sparing drug is needed for chronic or recurrent disease. The use of cyclosporine has been reported to be effective in problem or refractory cases. Finally, surgical débridement or grafting should be undertaken with great caution and only in patients who have no clinical evidence of active disease and are receiving appropriate immunosuppressive therapy.

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Drugs that suppress the activity of the immune system. They are given after organ transplantation to prevent the body's immune system causing tissue rejection. Some are also used in the treatment of autoimmune diseases (in which the body's immune system attacks the body's own tissues), including rheumatoid arthritis and myasthenia gravis. The immunosuppressants used to prevent transplant rejection include azathioprine, cyclosporin, mycophenolate mofetil, corticosteroids (such as prednisolone), and tacrolimus.

Because immunity is lowered during treatment with immunosuppressants, there is an increased susceptibility to infection (see bone marrow suppression), and regular blood counts may need to be carried out. Some drugs, especially the cytotoxic drugs used to treat cancer, cause immunosuppression as a side effect.

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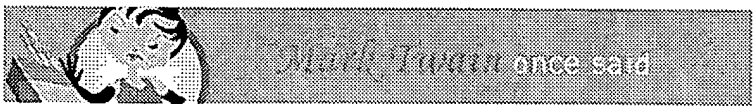

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an acute destructive ulcerating process of the skin, especially the legs. It may be associated with ulcerative colitis or Crohn's disease or with rheumatoid arthritis or other forms of arthritis affecting many joints. Treatment is with high doses of corticosteroids

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inflammation of one or more joints, characterized by swelling, warmth, redness of the overlying skin, pain, and restriction of motion. Over 200 diseases may cause arthritis, including rheumatoid arthritis, osteoarthritis, gout, tuberculosis, and other infections. Diagnosis is assisted by examination of the pattern of distribution of affected joints, X-rays, blood tests, and examination of synovial fluid obtained by aspiration of a swollen joint. **Mono-** or **oligoarthritis** is inflammation of one joint, **pauciarthritis** of a few (four or less), and **polyarthritis** of many joints, either simultaneously or in sequence. Any disease involving the synovial membranes or causing degeneration of cartilage may cause arthritis. Treatment of arthritis depends on the cause, but aspirin and similar analgesics are often used to suppress inflammation, and hence reduce pain and swelling. See also psoriatic arthritis, septic arthritis, haemarthrosis, pyarthrosis, hydrarthrosis. --**arthritic**

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arthr- (arthro-), prefix

arthrodic joint (gliding

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the second most common form of arthritis (after osteoarthritis). It typically involves the joints of the fingers, wrists, feet, and ankles, with later involvement of the hips, knees, shoulders, and neck. It is a disease of the synovial lining of joints; the joints are initially painful, swollen, and stiff and are usually affected symmetrically. As the disease progresses the ligaments supporting the joints are damaged and there is erosion of the bone, leading to deformity of the joints. Tendon sheaths can be affected, leading to tendon rupture. Onset can be at any age, and there is a considerable range of severity. Women are at greater risk. Rheumatoid arthritis is an autoimmune disease, and most patients show the presence of **rheumatoid factor** in their serum. There are characteristic changes on X-ray.

Treatment is with a variety of drugs, including anti-inflammatory analgesics, steroids, methotrexate, and gold injections. Surgical treatment is by excision of the synovium in early cases or by fusion or joint replacement once bony changes have occurred. (See also hip replacement.) The condition may resolve spontaneously, but is usually relapsing and remitting with steady progression. It may finally burn itself out,

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